SPONSOR

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REPORT FOR ACUTE TOXICITY TO FISH OF FRD902

(STATIC TEST)

Study No.: S2009NC031(s)-01

Report No.: R2009NC031(s)-01

Study Director: Shi Lili, professor

Date of Report Completion

Feb. 4, 2010

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STATEMENT OF GLP COMPLIANCE

Study No.: S2009NC031(s)-01

Report No.: R2009NC031(s)-01

According to "OECD Guidelines for testing of chemicals", "The guidelines for the testing of chemical (HJ/T 153-2004)"and "The guidelines of chemical testing good laboratory practices (HJ/T 155-2004)" issued by State Environmental Protection Administration (SEPA) of the People's Republic of China, this experiment was conducted under CMA (China Metrology Accreditation) and CNAS (China National Accreditation Service for Conformity Assessment) experimental conditions at our laboratory. The experimental protocol was strictly carried out in the process of the experiment, and the present report has reflected the experimental results truly and correctly.

Shu Lili — Feb. 4, 2010

(Study Director) — Date:

Shan Zhengjun — Feb. 4, 2010

(Laboratory Management) — Date:

QUALITY ASSURANCE STATEMENT

Study No.: S2009NC031(s)-01

Report No.: R2009NC031(s)-01

This experiment was carried out in accordance with the experimental protocol. It is hereby certified that what the present report describes has accurately reflected the raw data of the experiment.

During the on-site process, QAU Inspections were performed for this study. The dates of Quality Assurance inspection are given below.

Type of inspections	Phase/Process	Date	
		Review/Inspection	Reporting to SD
Study	Protocol Report	Dec. 8, 2009 Jan. 28, 2010	Dec. 8, 2009 Jan. 29, 2010
Process	Test solutions preparation Observation/Determination	Dec. 20, 2009 Dec. 20, 2009	Dec. 20, 2009 Dec. 20, 2009

Ge Feng

(Person responsible for QAU)

Feb. 4, 2010

Date:

STUDY DETAILS PAGE

Study number:	S2009NC031(s)-01
Report number:	R2009NC031(s)-01
Study title:	Acute Toxicity to Rare gudgeon (*Gobiocypris rarus*)
Test substance:	FRD902
Identity:	FRD902
Chemical name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)Propanoic acid, ammonium salt
Molecular formula:	$C_6 H F_{11} O_3 \cdot H_3N$
Molecular weight:	347.08
Lot number:	NA
Expiry date:	Jul. 2010
Appearance:	Liquid
Purity/Assay:	86.9%
Storage conditions:	Keep container tightly closed and store in a cool, dark, well ventilated location
Head of Department:	Shan Zhengjun
Study Director:	Shi Lili
Person attending to routine duties and technical queries in the temporary absence of the Study Director:	Wang Lei
Study Director contact details:	Shi Lili Telephone: 86 25 85287074 Facsimile: 86 25 85474630 Email: sll@nies.org sllnes@hotmail.com
Location of study:	Key Lab. of Pesticide Environmental Assessment and Pollution Control, MEP 8 Jiang-wang-miao Street, Nanjing 210042 Jiangsu, China
Study dates: Start: Completion: Draft report:	 Dec. 7, 2009 Jan. 28, 2010 Feb. 04, 2010

1 SUMMARY

Under static conditions, the acute toxicity of test substance (FRD902) to rare gudgeon (*Gobiocypris rarus*) was conducted according to the following guidelines: "The guidelines for the testing of chemicals", SEPA (HJ/T 153-2004); Procedure 203 of the "Guidelines for Testing of Chemicals" of the OECD: "Fish, Acute Toxicity Test" (1992) etc.

During the test period, the pH values of the control mediums and test mediums were between 7.21 and 7.41, and the Dissolved Oxygen (DO) values varied from 94.9% to 98.1% of the air saturation at the test temperature, and the total hardness was in the range of 138 mg ($CaCO_3$)/L to 141 mg ($CaCO_3$)/L. During the test, the temperature of the test mediums were maintained in the range of 22.8°C to 23.1°C, and all fishes in the control group were normal. With the same conditions, $K_2Cr_2O_7$ was used as the negative control substance, and the resulting 24 h-LC_{50} was 359 mg/L. So the study met the acceptability criteria prescribed by the protocol (pH: 6.0~8.5; dissolved oxygen concentration: > 60% of the air saturation value; total hardness: 10~250 mg ($CaCO_3$)/L; temperature: 23 °C±2 °C; 24 h-LC_{50} of $K_2Cr_2O_7$ in the range of 200 mg/L to 400 mg/L). Therefore the test was considered valid.

In order to confirm the stability of the test substance in the test medium, concentrations of the test samples during the Limit Test were analyzed by UPLC/MS. A linear regression equation was obtained with the peak area response (A) vs. the concentration of the test substance (c, mg/L): A =993180 c + 1799.9, with good linearity of r^2=0.994. The results show that linearity for the concentration range of 0.005 mg/L to 0.1 mg/L is good. Besides, the minimum detection amount of UPLC/MS for FRD902 is 5.0×10^{-11}g, and the minimum detection concentration for water sample is 0.005 mg/L. The analytical results showed that the concentration of the test substance is consistent in the test medium throughout the 96-hour test period (deviation within 20%). Thus a static test procedure was reasonable.

The results showed that under valid static test conditions, the maximum concentration causing no mortality and the 96 h-LC_{50} of the test substance to rare gudgeon are both greater than 150 mg/L (analysed concentration 145 mg/L). According to "The guidelines for the hazard evaluation of new chemical substances (HJ/T 154-2004)", FRD902 belongs to the category of low toxicity chemical for fish (*Gobiocypris rarus*).

2 INTRODUCTION

This test was designed to determine the acute toxicity of test substance (FRD902) to rare gudgeon (*Gobiocypris rarus*). The test fishes were exposed for 96 hours to the test solution. The study comprised at least one range-finding test followed by a definitive test to determine, where possible, the 96-hour median lethal concentration (LC_{50}) of the test substance, with 95% confidence limits. Where no toxicity was seen in the range-finding test, it would be followed by a Limit Test at a single concentration.

The study met the requirements of:

1) "The guidelines for the testing of chemicals", SEPA (HJ/T 153-2004).
2) HJ/T 154-2004.The guidelines for the hazard evaluation of new chemical substances. SEPA.
3) "The guidelines for the testing of chemicals", Beijing: China Environmental Sciences Publishing House. 2004. SEPA.
4) Part C.1 (Acute Toxicity for Fish) of EC Methods for the Determination of Ecotoxicity; - Annex to Council Regulation (EC), L142 (2008).
5) Procedure 203 of the 'Guidelines for Testing of Chemicals' of the OECD: "Fish, Acute Toxicity Test" (1992).
6) Ecological Effects Test Guidelines, OPPTS 850.1075, Fish Acute Toxicity Test, Freshwater and Marine. EPA 712-C-96-118, April 1996.

The Guide for Care and Use of Laboratory Animals (1988)

The in-life experimental procedures undertaken during the course of this study were subject to the provisions of the Guide for Care and Use of Laboratory Animals (1988) in China. The Guide, administered by the Ministry of Science and Technology of the People's Republic of China, regulates all scientific procedures in living animals which may cause pain, suffering, distress or lasting harm and provides for the designation of establishments where procedures may be undertaken, the licensing of trained individuals who perform the practical techniques and the issue of project licences for specified programmes of work.

This study complied with all applicable sections of the Guide and the associated Codes of Practice for the Housing and Care of Animals used in Scientific Procedures.

The number of animals used was the minimum that was consistent with scientific integrity and regulatory acceptability, consideration having been given to the welfare of individual animals in terms of the number and extent of procedures to be carried out on each animal.

3 EQUIPMENT & MATERIALS

3.1 Date of Test

Dec. 7, 2009 ~ Jan. 28, 2010

3.2 Test Organism

The test species of rare gudgeon, *Gobiocypris rarus*, were selected because of their known sensitivity to changes in water quality, availability throughout the year and ease of maintenance in the laboratory. They were obtained as fry from the Institute of Hydrobiology, Chinese Academy of Sciences in China. Details concerning the source of the fish were recorded in the raw data.

Upon receipt, fish were held for 12 days in holding tanks supplied with a continuous flow of aerated water before being used for testing. Fish used in the test were held for 7 days in water of the quality and temperature to be used in the test.

The wet-weight of the fish used for the test was approximately 0.3 g, with an average length of 2 - 3 cm (including tail).

During the holding period the tanks were inspected daily and any debris or unhealthy or dead fish removed.

Following a 48 hour settling-in period, mortalities were recorded and no dead fish was found.

A photoperiod of 12 hours light (light intensity: mean 1317 lux), provided by overhead fluorescent tubes, and 12 hours dark was maintained.

The fish were fed daily during the holding period with freeze dried brine shrimp from Golden Dragon Co., Ltd (TianJing, China). They were held without food for approximately 24 hours before being placed into the test vessels.

3.3 Dilution Medium

Good quality tap water which had been dechlorinated for at least 24 hours was used. The total hardness of the test water was about 140 mg ($CaCO_3$)/L, oxygen concentration at no less than 60% of the air saturation value and pH in the range of 7.2 ~ 7.5.

3.4 Apparatus

Normal laboratory apparatus and:

1) Oxygen meter (HITACHI HQ30 d. The Dissolved Oxygen can be directly read by immersing the probe in the water sample when the reading is stable.);
2) Thermometer; (HITACHI HQ30 d. The Temperature can be directly read by immersing the probe in the water sample when the reading is stable.);
3) Equipment for determination of hardness of water (Model: 16900, made by HITACHI company, the hardness can be directly read by immersing the probe in the water sample when the reading is stable.);
4) Tanks made of chemically inert material, with a sealable inert lid, and with a capacity of

approximately 8 L (Sanhe Zuping Glass Instrument Factory, Haimen);

5) Thermostatic water bath (Chang Yuan Medical Instrument Factory, Jiangsu);

6) ACQUITY R™ ultra Performance LC (Waters, USA)

4 TEST DESIGN

4.1 Preparation of the Test Solutions

The test solution of FRD902 was prepared by directly dissolving appropriate amount of FRD902 in dilution medium and then facilitating its dispersion by ultrasonication for 20 min. Details about the Preparations are showed as follows:

Test Type	Nominal Concentration (mg/L)	The Amount of The Test Substance Added (g)	Dilution Medium Volume (L)
Range-finding Test	1	0.005	5
	10	0.050	5
	100	0.500	5
Limit Test	100	0.500	5
	100	0.500	5
	100	0.500	5
	150	0.750	5
	150	0.750	5
	150	0.750	5

The test solutions were freshly prepared just before introduction of the test fish when the test started.

4.2 Observations and Evaluations

During the test, all kinds of abnormal responses of the fish observed would have been recorded, such as mortality, inactivity, strange swimming pattern, other abnormal behaviour, etc.. Fish would have been considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction.

4.3 Range-finding Test

The range-finding test, carried out under static conditions, was regularly conducted to determine the range of concentrations for the subsequent test.

In the range-finding test, groups of fish (5 per group) were exposed to three widely-spaced concentrations of the test substance (1, 10, 100 mg/L). Synchronously one control group was conducted in test water without the test substance. For each test tank (Total volume: 8 L) 5 L test solution was filled in. No replicates were used.

The test fish should be randomly chosen and put in different test solutions after the temperature has been adjusted to the required value. This should be done in 30 min.

During the test, the following conditions were maintained:

-Light: 12 hours photoperiod daily (light intensity: 1000 to 1500 lux);

-Temperature: (23 ± 2)°C for rare gudgeon;

-Oxygen concentration: at least > 60% of the air saturation;

-Feeding: none.

Test duration was 96 hours. Once the test started, the mortalities of the fish were recorded at 24-h, 48-h, 72-h and 96-h, and then the maximum concentration causing no mortality and the minimum concentration causing 100% mortality were determined. Dead fish would be removed at least once daily and discarded.

4.4 Limit Test

Static method was adopted in limit test according to the stability of the test solution indicated by Table 1. Based on the results of the range-finding test, test concentrations of 100 mg/L and 150 mg/L were assigned in limit test. Synchronously one control group was used in the test. Three replicates were assigned for each treatment group and control group, while the initial number of testing fish was 10 for each replicate.

The test condition was maintained as same as the range-finding test. At 24-h, 48-h, 72-h and 96-h, the mortalities of the fish were recorded, and observations on individual behaviour were performed.

Meanwhile, measurements of pH, dissolved oxygen and temperature were carried out and recorded daily.

4.5 Validity of Test

(1) Control group

A control group, comprising the same number of fish as that exposed at each test concentration, was placed into test water alone.

(2) Reference substance test

With the conditions maintained as before, $K_2Cr_2O_7$ should be used as the test substance and the resulting 24 h-LC_{50} should be in the range of 200 to 400 mg/L. Otherwise, the test conditions was regarded as invalid.

(3) Fish loading

The aquaria were of an adequate size to meet the criterion of a maximum loading of 1 g fish per litre of test medium.

(4) Validity of test result

For the test to be valid, mortality in the control group must not exceed 10% or one fish per group if less than ten are used at the end of the test. The concentration of dissolved oxygen must be more than 60% of the air saturation value during the test and exposure concentrations should be maintained throughout the test (within 80% of the initial concentration); where this is not possible, results should be based on the measured concentration.

4.6 Stability of Test Solution and Chemical Analysis

(1) Preparation of standard storck solution

The standard stock solution I of FRD902 (1000 mg/L) for the calibration curve was prepared by dissolving 0.0576 g of the test substance into 50 mL deionised water.

The standard stock solution II of FRD902 (10 mg/L) for the calibration curve was prepared by drawing 1.0 mL stock solution I (1000 mg/L) and diluting it to 100 mL with deionised water.

The standard stock solution III of FRD902 (1 mg/L) for the calibration curve was prepared by drawing 1.0 mL stock solution I (10 mg/L) and diluting it to 10 mL with deionised water.

(2) Working solution

Prepare the standard working solution of the test substance at 0.005 mg/L, 0.01 mg/L, 0.02 mg/L, 0.05 mg/L and 0.1 mg/L by diluting the standard stock solution III of FRD902 (1mg/L) with deionised water. Details of the solutions are showed as follows:

Concentration (mg/L)	The Concentration of the Storage Solution Added (mg/L)	The Volume of the Storage Solution Added (mL)	Final Volume after Dilution (mL)
0.005	1	0.05	10
0.01	1	0.1	10
0.02	1	0.2	10
0.05	1	0.5	10
0.1	1	1	10

(3) UPLC/MS conditions

Apparatus: ACQUITY R™ ultra Performance LC, Quattro Premier XE MS-MS (Waters)

Column: ACQUITY UPLC® BEH C18 1.7 μm, 2.1×50 mm (Waters)

Mobile phase: Methanol: water=60:40 (v : v)

Column Temperature: 30 °C

Flow rate: 0.3 mL/min

Injection volume: 10 μL

MS detection parameters are as follows:

Data type: SIR data

Ionisation mode: Electrospray

Polarity: negative

Capillary: 3.0 kV

Extractor: 3.0 V

RF Lens: 0.2 V

Source Temperature: 110 °C

Desolvation Temperature: 380 °C

Cone Gas Flow: 50 L/h

Desolvation Gas Flow: 500 L/h

LM Resolution 1: 7.9

LM Resolution 2: 5.1

HM Resolution 1: 15.0

HM Resolution 2: 14.7

Ion Energy 1: 0.4

Ion Energy 2: 2.0

Collision Gas (Pressure): $3.43e^{-3}$ mbar

Ion (m/z)	Dwell (s)	Cone Volt. (V)
284.76	0.100	-31

Under the above conditions, the retention time of FRD902 was about 0.66 min (see Fig. 2).

(4) Sampling and analysis of the test solution

Samples were taken (at least in duplicate) from each concentration on five occasions during the limit test (at 0 h, 24 h, 48 h, 72 h and 96 h). On each occasion, one sample was analysed after filtration by 0.22 μm millipore filter; the remaining samples were retained in case further analysis would be required. The filtrates obtained were diluted 10000-fold with deionised water and then analysed by UPLC/MS.

5 DATA PROCESSING

Korbor processing method may be used to calculate the LC_{50} and LC_{50} confidence limits.

(1) LC_{50} calculate formula

$$\log(LC_{50}) = X_m - i(\sum P - 0.5) \quad \text{.....(1)}$$

Where, LC_{50}—the concentration leading to 50% mortality of the fish (mg/L);

X_m—logarithm of highest concentration;

i—logarithm of two concentration that is next;

∑P—sum of all treatment mortality (expressed as decimal).

(2) LC_{50} Calculate confidence limits (P=0.95) formula

$$\text{SD: } S\log(LC_{50}) = i\sqrt{\sum \frac{pq}{n}} \quad \text{.....(2)}$$

Where, p—mortality in one treatment;

q—1-p;

i—logarithm of two concentration that is next;

n—number of fishes in every treatment.

$$\text{Confidence limits (P = 0.95) of } \log(LC_{50}) = \log(LC_{50}) \pm 1.96S\log(LC_{50}) \quad \text{.....(3)}$$

As a limit test was carried out, no statistical analysis was used for test substance in this study.

6 RESULTS

6.1 Analytical Method for Determination of FRD902 in Water

(1) Calibration curve

A series of standard solutions with concentration at 0.005 mg/L, 0.01 mg/L, 0.02 mg/L, 0.05 mg/L and 0.1 mg/L were measured under the UPLC/MS conditions mentioned above. Based on the test result, a linear regression equation was obtained between the concentration and the area of the peak emerged at 0.66 min: A =993180 c + 1799.9, with good linearity of r^2=0.994, where A represents peak area; and c is the concentration of the test substance (mg/L) (See Figure 1). The results show that linearity for concentration range of 0.005 mg/L to 0.1 mg/L is good.

(2) Detection limit

The minimum detection amount of UPLC/MS for FRD902 is 5×10^{-11}g, and the minimum detection concentration for water sample is 0.005 mg/L.

6.2 Analysis of FRD902 in Test Solutions

The analyzed results for the test samples from the limit test are given in Table 1. Figure 3, Figure 4 and Figure 5 showed the chromatographs of the control sample and the treated sample (100 mg/L and 150 mg/L) from the limit test. The results indicated that concentration of FRD902 was

stable (within 80% of the initial concentration) in the water during the test period. Thus static method used in the limit test was reasonable.

6.3 Test Condition

During the Limit test, the pH, dissolved oxygen concentration, total hardness and temperature of the control and treatment groups were showed in Table 2.

During the whole test period, the pH values of the control mediums and test mediums were between 7.21 and 7.41, and the Dissolved Oxygen (DO) values varied from 94.9% to 98.1% of the air saturation at the test temperature, and the total hardness was in the range of 139 mg ($CaCO_3$)/L to 141 mg ($CaCO_3$)/L. During the test, the temperature of the test mediums were maintained in the range of 22.8°C to 23.1°C, and all fishes in the control group were normal. With the same conditions, $K_2Cr_2O_7$ was used as the negative control substance, and the resulting 24 h-LC_{50} was 359 mg/L (Table 3). So the study met the acceptability criteria prescribed by the protocol (pH: 6.0~8.5; dissolved oxygen concentration: > 60% of the air saturation value; total hardness: 10~250 mg ($CaCO_3$)/L; temperature: (23 ±2) °C; 24 h-LC_{50} of $K_2Cr_2O_7$ in the range of 200 to 400 mg/L). Therefore the test was considered valid.

6.4 Mortality and Effects

Table 4 and Table 5 show the mortality data during the range-finding test and Limit Test respectively. Table 6 showed the observations during the Limit test. All fish in the control and the treatment were alive and appeared normal.

6.5 Conclusions

The results showed that under static conditions, the maximum concentration causing no mortality and the 96 h-LC_{50} of the test substance to rare gudgeon are both greater than 150 mg/L (analysed concentration 145 mg/L).

96h-LC_{50} > 150 mg/L (analysed concentration: 145mg/L);

96h-NOEC > 150 mg/L (analysed concentration: 145 mg/L).

According to "The guidelines for the hazard evaluation of new chemical substances (HJ/T 154-2004)", FRD902 belongs to the category of low toxicity chemical for rare gudgeon (*Gobiocypris rarus*).

7 HEALTH & SAFETY

In order for PEAPC to comply with Law of the People's Republic of China on the Prevention and Treatment of Occupational Diseases 2001, and the current Control of Substances Hazardous to Health Regulations, it is a condition of undertaking the study that the Sponsor provide PEAPC with all information available to it regarding known or potential hazards associated with the

handling and use of any substance supplied by the Sponsor to PEAPC. The Sponsor also complied with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed PEAPC test substance data sheet must be received at PEAPC before the test substance can be handled in the laboratory.

8 MAINTENANCE OF RECORDS & DOCUMENTATION

All raw data arising from the performance of this study will remain the property of the Sponsor.

Records and documentation relating to this study (including electronic records) will be maintained in the archives of PEAPC for a period of five year from the date on which the Study Director signs the final report. This includes the Study Protocol, raw data, a copy of the final report.

Types of sample which are unsuitable, by reason of instability, for long term retention and archiving will be disposed of after the periods stated in PEAPC Standard Operating Procedures. Test substance remaining will be retained by PEAPC in its archive for a period of one year from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials.

If requested, PEAPC will continue to retain the materials subject to a reasonable fee as agreeable to the Sponsor.

PEAPC will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

9 REFERENCE

1) OECD. 203 Fish, Acute Toxicity Test. Paris: 17 July 1992.

2) SEPA. The guidelines for the testing of chemicals. HJ/T 153-2004. 2004.

3) SEPA. The guidelines for the testing of chemicals. Beijing: China Environmental Sciences Publishing House. 2004.

4) Part C.1 (Acute Toxicity for Fish) of EC Methods for the Determination of Ecotoxicity; - Annex to Council Regulation (EC), L142 (2008).

5) Ecological Effects Test Guidelines, OPPTS 850.1075, Fish Acute Toxicity Test, Freshwater and Marine. EPA 712-C-96-118, April 1996.

6) SEPA. The guidelines for the hazard evaluation of new chemical substances. HJ/T 154-2004. 2004.

TABLES

Table 1 Stability Test Results of FRD902 in Test Medium (Limit Test).

Nominal Concentration (mg/L)	Analysed Concentration (mg/L)				
	0 h	24 h	48 h	72 h	96 h
0	ND①	ND	ND	ND	ND
100	117	110	112	111	110
150	145	146	147	142	143

①ND: not detected.

Table 2 Water Quality Parameters of Test Solutions during the Limit Test

Nominal Concentration (mg/L)	Duration(h)	pH	Temperature (°C)	Dissolved oxygen (%)	Hardness (mg/L$CaCO_3$)
0	0	7.32	23.0	98.1	140
	24	7.30	23.1	97.5	140
	48	7.30	22.8	96.2	140
	72	7.32	23.0	95.5	141
	96	7.33	23.1	94.3	140
100	0	7.41	23.0	97.6	140
	24	7.38	23.1	96.5	141
	48	7.26	23.0	96.4	139
	72	7.30	22.9	95.3	140
	96	7.21	23.0	95.0	140
150	0	7.41	23.0	97.5	140
	24	7.37	23.1	96.4	140
	48	7.31	23.0	95.3	139
	72	7.30	23.0	94.9	141
	96	7.29	22.9	95.0	140

Table 3 Toxicity of Potassium Dichromate to *Gobiocypris rarus*

Nominal Concentration (mg/L)	Initial Number of Fish	The Number of the Dead Fish			
		6 h	12 h	18 h	24 h
0	7	0	0	0	0
	7	0	0	0	0
	7	0	0	0	0
100	7	0	0	0	0
	7	0	0	0	0
	7	0	0	0	0
200	7	0	0	0	1
	7	0	0	1	1
	7	0	0	0	0
300	7	0	1	1	2
	7	0	0	1	1
	7	0	1	2	2
400	7	1	3	4	4
	7	0	2	2	3
	7	1	2	3	4
500	7	1	3	4	6
	7	2	4	6	7
	7	2	3	5	7
LC_{50} (mg/L)		—	—	421	359
95% confidence limit (mg/L)		—	—	392 ~ 452	341 ~ 379

Table 4 Mortality during the Range-finding Test

Nominal Concentration (mg/L)	Initial Number of Fish	The Number of the Dead Fish			
		24 h	48 h	72 h	96 h
0	5	0	0	0	0
1	5	0	0	0	0
10	5	0	0	0	0
100	5	0	0	0	0

Table 5 Mortality during the Limit Test

Nominal Concentration (mg/L)	Analysed Concentration① (mg/L)	Initial Number of Fish	The Number of the Dead Fish			
			24 h	48 h	72 h	96 h
0	ND②	10	0	0	0	0
		10	0	0	0	0
		10	0	0	0	0
100	118	10	0	0	0	0
		10	0	0	0	0
		10	0	0	0	0
150	145	10	0	0	0	0
		10	0	0	0	0
		10	0	0	0	0

①The average of the analysed concentrations at 0-h, 24h-, 48-h, 72-h, 96-h.
②ND: not detected.

Table 6 Visual Observations during Limit Test

Nominal Concentration (mg/L)	No.	Visual Observations			
		24 h	48 h	72 h	96 h
0	#1	normal	normal	normal	normal
	#2	normal	normal	normal	normal
	#3	normal	normal	normal	normal
100	#1	normal	normal	normal	normal
	#2	normal	normal	normal	normal
	#3	normal	normal	normal	normal
150	#1	normal	normal	normal	normal
	#2	normal	normal	normal	normal
	#3	normal	normal	normal	normal

FIGURE

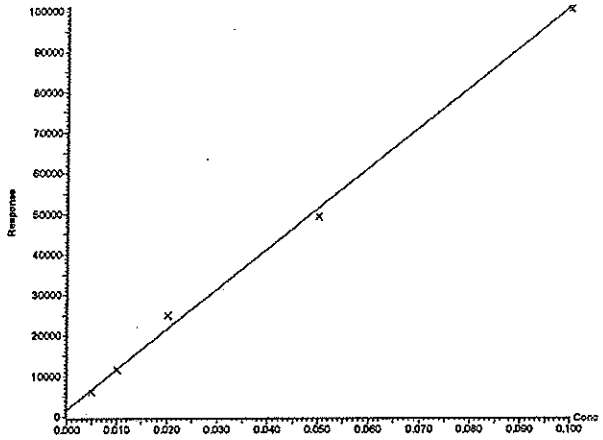


Figure 1 Calibration Curve for the Test Substance

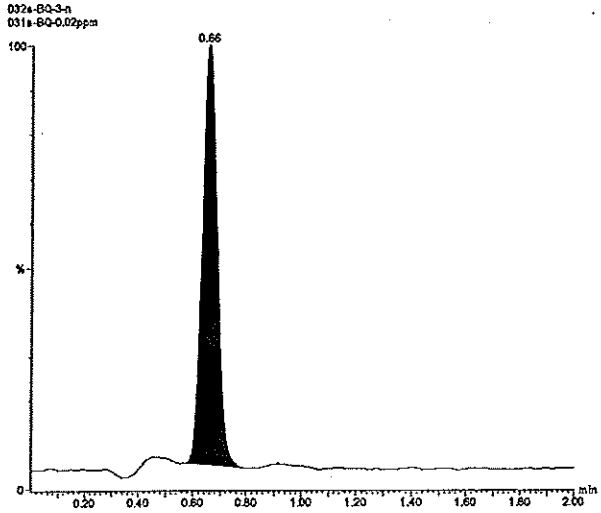
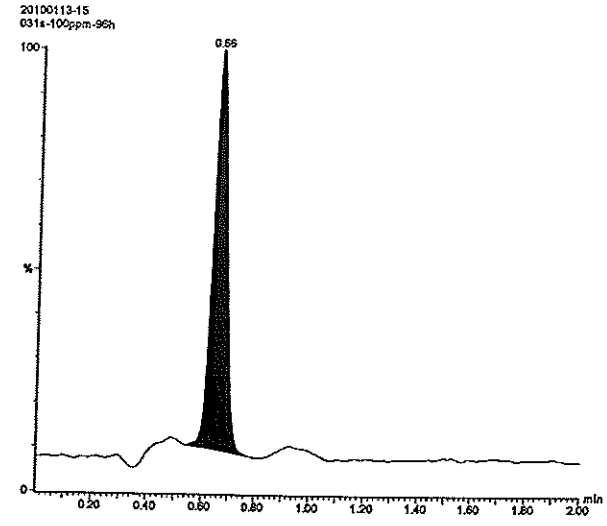
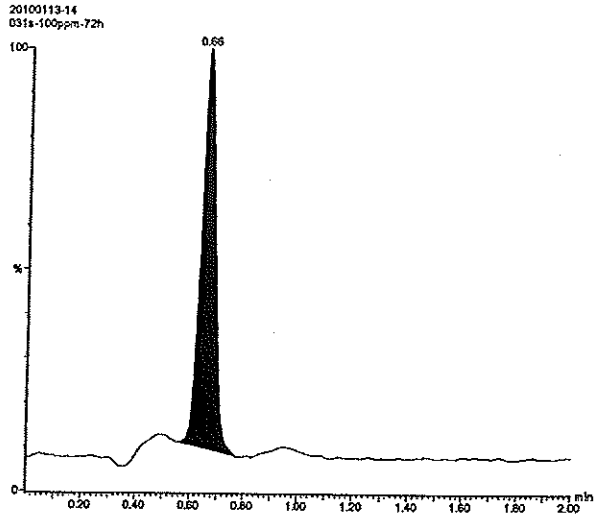


Figure 2 Standard UPLC/MS Chromatogram of the Test Substance (0.02 mg/L)

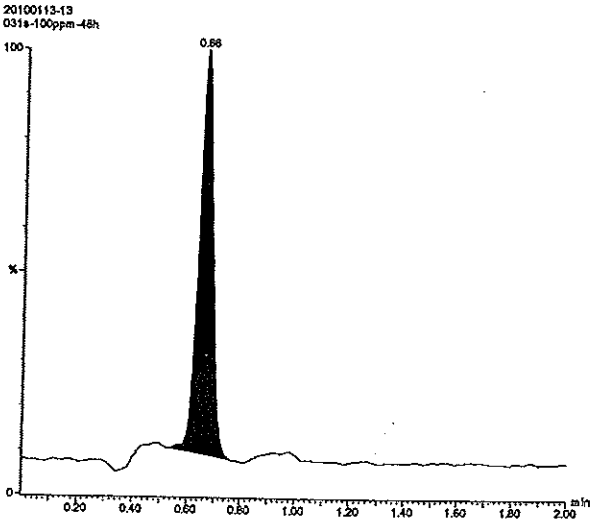
Figure 3 UPLC/MS Chromatogram of the Control Sample (Limit Test)



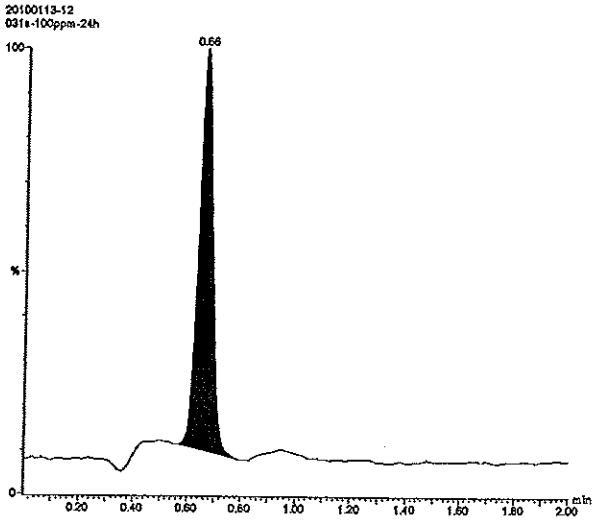
96 h



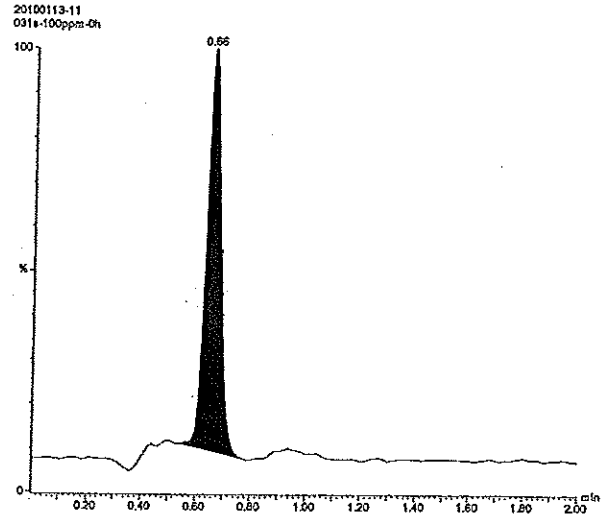
72 h



48 h

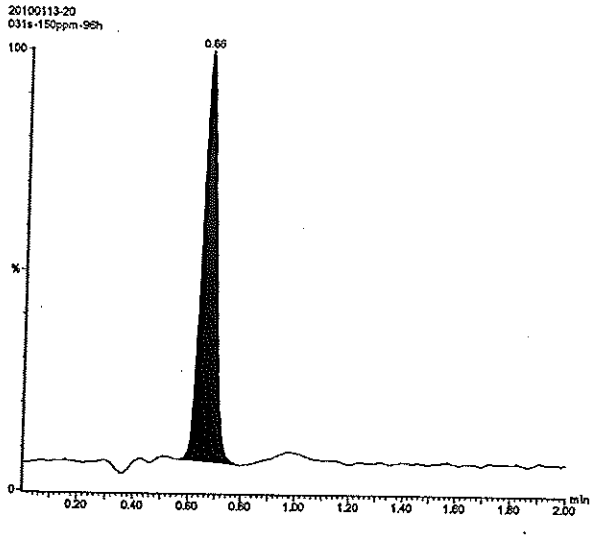


24 h

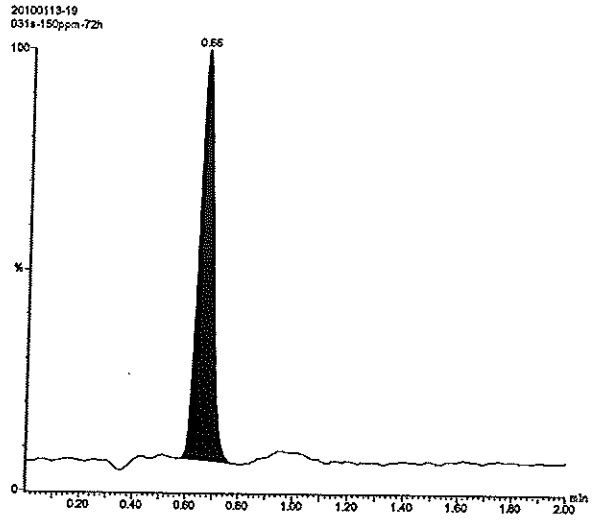


0 h

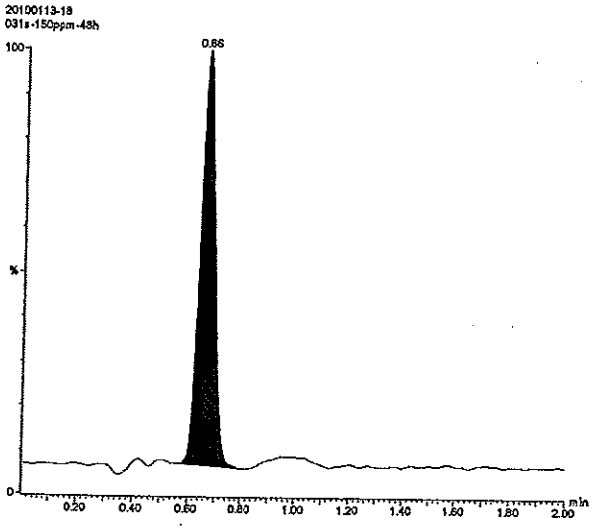
Figure 4 UPLC/MS Chromatograms of the Test Substance in Limit Test (100 mg/L)



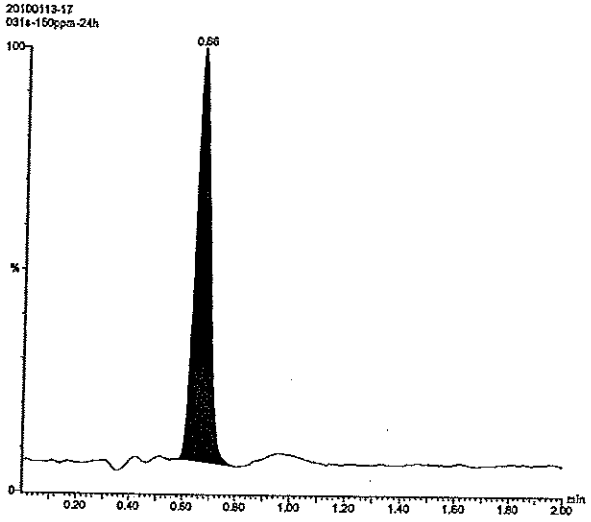
96 h



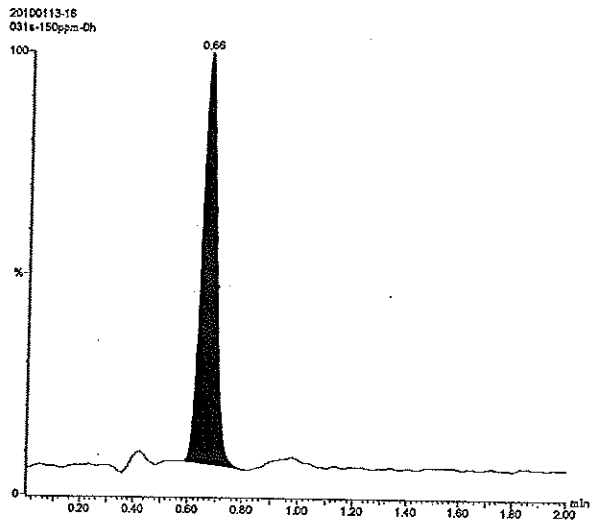
72 h



48 h



24 h



0 h

Figure 5 UPLC/MS Chromatograms of the Test Substance in Limit Test (150 mg/L)